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Review

A minireview on the *in vitro* and *in vivo* experiments with anti-*Escherichia coli* O157:H7 phages as potential biocontrol and phage therapy agents

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ABSTRACT

Phage therapy is an old method of combating bacterial pathogens that has recently been taken into consideration due to the alarming spread of antibiotic resistance. *Escherichia coli* O157:H7 is a foodborne pathogen that causes hemorrhagic colitis and life-threatening Hemolytic Uremic Syndrome (HUS). There are several studies on isolation of specific phages against *E. coli* O157:H7 and more than 60 specific phages have been published so far. Although *in vitro* experiments have been successful in elimination or reduction of *E. coli* O157:H7numbers, *in vivo* experiments have not been as promising. This may be due to escape of bacteria to locations where phages have difficulties to enter or due to the adverse conditions in the gastrointestinal tract that affect phage viability and proliferation. To get around the latter obstacle, an alternative phage delivery method such as polymer microencapsulation should be tried. While the present time results are not very encouraging the work should be continued as more efficient phage treatment regimens might be found in future.

Contents

1. Introduction

Although first indications of bacteriophages (bacterial viruses) were reported already in 1896 (Hankin, 1896), bacteriophages were first time isolated in early 1900's (d'Herelle, 1917; Twort, 1915). Phages were used to treat infectious diseases in different parts of the world until late 1930s. The advent of antibiotics, improper use of phages and unstable formulations of phage particles reduced the use of phage therapy especially in Western countries (Bradbury, 2004). In recent years, the emergence of antibiotic resistance in bacterial populations has encouraged researchers to find bacteriophages to combat bacterial infections (Deresinski, 2009).

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Escherichia coli O157:H7 that is present in the normal flora of livestock (Paton and Paton, 1998) can cause hemorrhagic colitis and life-threatening HUS (Hemolytic Uremic Syndrome). Outbreaks of this pathogen can originate from meat, milk, vegetables and water (Ferens and Hovde, 2011). There is a controversy about using antibiotics in treatment of *E. coli* O157:H7 infections as it is believed that use of certain antibiotics could induce the release of shiga toxins (Galland et al., 2001). Clinical studies have demonstrated that antibiotic treatment can indeed increase the risk of HUS development (Wong et al., 2012). In addition, antibiotic resistance among *E. coli* O157 isolates has increased. In one study, 21% of human clinical isolates of *E. coli* O157 were resistant to ampicillin, 12% to sulfamethoxazole, 15% to cephalothin, 12% to tetracycline, and 5% to trimethoprim-sulfamethoxazole (Schroeder et al., 2002).

Specific phages for *Escherichia coli* O157:H7 can be suitable candidates either for infection prevention or treatment of infected people.

2. *E. coli* O157 phages

More than 60 phages specific for *E. coli* O157:H7 have been reported (Table 1). Most of these phages (27) belong to *Myoviridae* family. Eleven phages belong to *Siphoviridae* and only 3 phages are from *Podoviridae* family. The remaining phages have not been classified yet. Sixteen of these phages have full genomic sequences submitted to NCBI.

3. Prevention of infection

Numbers of *E. coli* O157:H7 bacteria can be reduced by application of phages on surfaces or addition of phages to animal hides or bodies. This can decrease the numbers of bacteria in foodstuffs and thereby prevent human infections. Experimental *in vitro* studies to reduce bacterial numbers on surfaces by the use of anti-O157:H7 phages are presented in Table 1 and discussed below.

3.1. Application of phages on surfaces and to foodstuffs

Several studies have demonstrated the usefulness of phage application on post-slaughter meat, on working surfaces and to vegetables (Table 1).

A phage cocktail of pp01, e11/2, and e4/1c phages eliminated efficiently *E. coli* O157 from meat when incubated at 37 °C. For this, 2×10^2 *E. coli* cells were spotted on meat portions. After an hr, 2×10^8 PFU phage particles were spotted onto it (O'Flynn et al., 2004). Hong et al. also used a cocktail of 3 phages (FFH1, FFH2, and FFH3) at multiplicity of infection (MOI) of 1 on contaminated ground beef, spinach and cheese contaminated with 10^7 CFU/mL *E. coli* O157:H7 bacteria. The results showed that the treatment was effective in reducing bacterial numbers in meat by almost 2 logs and in spinach by over 3 logs when stored 24 h at room temperature, but not in cheese (Hong et al., 2014).

Stainless steel coupons were immersed in 30 mL of 10^8 CFU/mL of *E. coli* O157 suspension, and the coupons with an average of 4×10^2 CFU attached cells were then immersed in a phage KH1 suspension of 5×10^7 PFU/mL. This approach reduced the numbers of *E. coli* by 1.2 logs during a 1 day exposure (Sharma et al., 2005). Viazis et al. tested a cocktail of 8 phages (38, 39, 41, AR1, 42, CEV2, ECB7, ECA1) on stainless steel, ceramic tile, and polyethylene chips. The treatment could significantly reduce bacterial concentrations on these surfaces. They also treated spinach and lettuce with the cocktail alone and in combination with *trans*-cinnamaldehyde. Although the cocktail was effective in reducing *E. coli* O157:H7 counts on vegetables, the combination was much more effective and killed bacteria completely. These experiments were carried out by spotting 10^6 PFU phage cocktail on the dried *E. coli* O157:H7 spots with 10^4–10^6 CFU (Viazis et al., 2011a, 2011b). The contaminated stainless steel, ceramic and plastic sheets were treated with phage PhaxI and showed that the treatment reduced the *E. coli* O157 contamination by 95–98% (Table 1). PhaxI also killed *E. coli* O157 in milk during a 90 minute incubation (Shahrbabak et al., 2013).

Magnone et al. used EcoShield, SalmoFresh, and ShigActive to control *E. coli* O157:H7, *Salmonella*, and *Shigella* spp. on fresh fruits and vegetables (Magnone et al., 2013). Ecoshield™ (ECP-100) is a FDA-cleared commercial phage cocktail of ECML-4, ECML-117, and ECML-134 bacteriophages and it is used to eliminate or reduce food contamination of *E. coli* O157:H7 (Carter et al., 2012; Ferguson et al., 2013). Spraying EcoShield (1×10^6 to 5×10^6 PFU/g) reduced *E. coli* numbers by 94% and 87% in beef and lettuce with an *E. coli* contamination of about 10^3 CFU/g, respectively, during a 5 min contact time (Carter et al., 2012). Boyacioglu achieved a *ca.* 2.5 log CFU/cm^2 reduction of bacterial numbers (4.5 log CFU/cm^2) on lettuce and spinach by spraying EcoShield (3×10^6 PFU/cm^2) together with a modified atmosphere packaging (Boyacioglu et al., 2013). Spray application of EcoShield with high phage concentrations (10^{10} and 10^9 PFU/mL) significantly reduced *E. coli* numbers in contaminated hard surfaces and different food samples (Abuladze et al., 2008). Immersion of lettuce in 6×10^9 PFU/mL EcoShield for 2 min followed by spot inoculation with 1.2×10^5 CFU/mL (2.5×10^2 CFU/cm^2) of *E. coli* O157:H7 was significantly effective in reduction of the pathogen. However, the low titer phage suspension (2×10^8 PFU/mL) was not as effective. Spraying 2×10^9 PFU/mL EcoShield after inoculation of 8×10^6 CFU/mL *E. coli* O157:H7 was even more effective than immersion (Ferguson et al., 2013). EcoShield (10^8 PFU/cm^2) was also effective in reducing bacterial numbers on fresh-cut cantaloupes (pipetted phage) and lettuce (sprayed phage) at 4 °C inoculated with 6×10^3 CFU/cm^2 *E. coli* O157:H7 (Sharma et al., 2009). A cocktail of six *E. coli* O157 specific phages isolated from feedlot by Callaway and coworkers were used by spraying 50 mL of 10^8 PFU/mL suspension on spinach harvester blades contaminated with 7×10^4 *E. coli* O157 bacteria; this treatment was able to kill bacteria on blades during a 2 h incubation (Patel et al., 2011).

In all studies, the anti-O157:H7 coliphages were effective in eliminating or reducing contamination by this pathogen on surfaces of food and material. However, the rate of elimination was affected by the time and temperature of the incubation and the relative numbers of *E. coli* O157:H7 and the bacteriophages.

3.2. Biocontrol or pre-harvest experiments

Some studies have applied phages to control *E. coli* O157:H7 numbers in live animals or hides. Theoretically this could decrease the spreading of the pathogen onto food and water, but it has proven to be less efficient than application of phages on surfaces and vegetables. While it has been convincingly demonstrated that cattle functions as a major *E. coli* O157:H7 reservoir, the bacterial shedding is periodic with the maximum incidence during summer (Bach et al., 2003). Therefore, to circumvent the potential problems caused by the erratic nature of the pathogen shedding, many experiments were performed on animals negative for *E. coli* O157:H7 in feces (Bach et al., 2003; Callaway et al., 2008; Rozema et al., 2009).

In one study performed with phage DC22 only an extremely high MOI (10^5 PFU/CFU) could decrease the *E. coli* O157:H7 levels (Table 1). The experiment was performed in Rusitec (an artificial rumen system) where an inoculation of phage at a MOI of 10^5 PFU/CFU could eliminate10^7 CFU of *E. coli* O157:H7 from the system within 4 h, although an inoculation of the phage at the same MOI was not effective in reducing 10^8 CFU of *E. coli* O157:H7 bacteria in sheep feces (Table 1). Furthermore, in both treated and control lamb groups, the O157:H7 numbers had decreased significantly after 13 days and completely eliminated one month post inoculation (Bach et al., 2003).

Raya et al. showed that a single oral dose of phage CEV1 (~10^{11} PFU) could reduce the level of *E. coli* O157:H7 (~10^{10} CFU by oral gavage) by 2 logs in ruminal, cecal, and rectal contents of sheep, demonstrating that in this case the phage was a promising candidate for phage therapy (Raya et al., 2006). Raya and coworkers also reported a significant

Table 1
Phages against *Escherichia coli* O157:H7used in *in vivo* and *in vitro* experiments.

Phage (family)	Infection model	Number of bacteria		Efficacy	Reference
		Before	After		
***In vitro* experiments**[a]					
DC22 (*Myoviridae*)	Artificial rumen system (Rusitec)	10^7	Undetectable	100% after 4 h	(Bach et al., 2003)
CEV2 (*Siphoviridae*)	Vegetables, stainless steel, ceramic, polyethylene chips (in a cocktail)	10^4–10^6	Undetectable	100%	(Viazis et al., 2011a, 2011b)
38, 39, 41, 42, ECA1, ECB7 (?)	Vegetables, stainless steel, ceramic, polyethylene chips (in a cocktail)	10^4–10^6	Undetectable	100%	(Viazis et al., 2011a, 2011b)
AR1 (*Myoviridae*)	Vegetables, stainless steel, ceramic, polyethylene chips (in a cocktail)	10^4–10^6	Undetectable	100%	(Viazis et al., 2011a, 2011b)
KH1 (?)	Stainless steel	4×10^2	4×10^1	Partial	(Sharma et al., 2005)
PhaxI (*Myoviridae*)	Milk	1.5×10^5	Undetectable	Efficient	(Shahrbabak et al., 2013)
	Stainless steel, ceramic and plastic sheet	5×10^5	Undetectable	Efficient	Unpublished data
pp01 (*Myoviridae*)	Meat, combined with e11/2 and e4/1c	2×10^2	Undetectable	Efficient	(O'Flynn et al., 2004)
e11/2 (112) (*Myoviridae*)	Meat as above	2×10^2	Undetectable	Efficient	(O'Flynn et al., 2004)
	Cattle hide	10^6 CFU/cm^2	~10^4 CFU/cm^2	Almost efficient	(Coffey et al., 2011)
	Model rumen system	10^3 and 10^6 CFU/ml	10^1	Efficient	(Rivas et al., 2010)
e4/1c (*Siphoviridae*)	Meat as above	2×10^2	Undetectable	Efficient	(O'Flynn et al., 2004)
	Cattle hide as above	10^6 CFU/cm^2	~10^4 CFU/cm^2	Almost efficient	(Coffey et al., 2011)
	Model rumen system	10^3 and 10^6	1.5×10^2–10^3	Efficient	(Rivas et al., 2010)
ECML-4, ECML-117, ECML-134 (*Myoviridae*)	Hard surfaces	10^5			Low
	Vegetables,	10^4			Low
	Ground beef	10^6			Low
	Beef, lettuce	~10^3 CFU/g			Less than 10^3
	Lettuce			2.5×10^2 CFU/cm^2	Undetectable
	Lettuce, spinach			3×10^4 CFU/cm^2	~10^2 CFU/cm^2
Efficient	(Abuladze et al., 2008)				
Efficient	(Carter et al., 2012)				
Efficient	(Ferguson et al., 2013)				
Efficient	(Boyacioglu et al., 2013)				
Efficient					
Efficient					
Callaway phages (?)	Stainless steel	7×10^4	Undetectable	Efficient	(Patel et al., 2011)
FFH1, FFH3 (*Siphoviridae*)	Ground beef, spinach	10^7	~10^5	Efficient	(Hong et al., 2014)
	Cheese (in combination with FFH2)	10^7	10^7	Not efficient	
FFH2 (*Myoviridae*)	Ground beef, spinach	10^7	~10^5	Efficient	(Hong et al., 2014)
	Cheese (in combination with FFH1, FFH3)	10^7	10^7	Not efficient	
P2BH2, PAH6 (?)	Rabbit ileal loop	~10^8	~10^2	Efficient	(Alam et al., 2011)
FAHEc1 (*Myoviridae*)	Beef	1.4×10^4	Undetectable	Efficient	(Hudson et al., 2013)
***In vivo* experiments**					
DC22 (*Myoviridae*)	Sheep, orally	10^8	Undetectable (after 1 month)	No difference with control	(Bach et al., 2003)
CEV1 (*Myoviridae*)	Sheep, orally alone or in combination with CEV2	~10^{10} 10^{10}	Less than10^4 (in cecum) ~8×10^2	2–3 log more reduction compared to control 99% alone, 99.9% cocktail	(Raya et al., 2006) (Raya et al., 2011)
CEV2 (*Siphoviridae*)	Sheep, orally in combination with CEV1	10^{10}	~8×10^2	99.9%	(Raya et al., 2011)
SH1 (?)	Mice, orally alone or in combination with KH1	10^8 10^6	Undetectable 2.5×10^2	Completely efficient Partially efficient	(Sheng et al., 2006)
	Steer, rectally in combination with KH1				
KH1 (?)	Sheep, orally alone	3.5×10^{10}	Similar to untreated	Not efficient	(Sheng et al., 2006)
	Mice and steer in combination with SH1	Such as SH1	Such as SH1	Such as SH1	
e11/2 (112) (*Myoviridae*)	Cattle, orally in combination with e4/1c	10^{10}	About 10^3 CFU/g feces	Not efficient compared to control	(Rivas et al., 2010)
e4/1c (*Siphoviridae*)	Cattle, orally in combination with e11/2	10^{10}	About 10^3 CFU/g feces	Not efficient compared to control	(Rivas et al., 2010)
Callaway phages (?)	Sheep, orally	10^{10}	~10^4 CFU/g feces	Efficient compared to control	(Callaway et al., 2008)
SP21, SP22 (?)	Mice, orally (cocktail with SP15)	10^9	Less than 10^2 CFU/g feces	Efficient (daily phage administration)	(Tanji et al., 2005)
SP15 (*Syphoviridae*)	Mice, orally (cocktail with SP21 and SP22)	10^9	Less than 10^2 CFU/g feces	Efficient (daily phage administration)	(Tanji et al., 2005)
rV5, wV7, wV8, wV11 (*Myoviridae*)	Steer, oral and rectal	5×10^{10}	10^2–10^3 CFU/g feces (day 12)	Generally not efficient compared to non-treated control	(Rozema et al., 2009)
ΦD, ΦW (*Myoviridae*)	Mice, injection alone, orally (ΦD)	10^7	0 (for ΦD), ~10^5 (for ΦW)	Efficient after 48 h	(Capparelli et al., 2006)
Phage with no reported experiments					
LG1 (*Myoviridae*)	–	–	–	–	(Goodridge et al., 2003)
CBA65 (?)	–	–	–	–	(Viazis et al., 2011a,

Table 1 (*continued*)

Phage (family)	Infection model	Number of bacteria		Efficacy	Reference
		Before	After		
					2011b, 2011c)
SP09 (?)	–	–	–	–	(Tanji et al., 2004)
JS98 (*Myoviridae*)	–	–	–	–	(Zuber et al., 2007)
phiKP26 (*Siphoviridae*)	–	–	–	–	(Amarillas et al., 2013)
EC6 (*Myoviridae*)	–	–	–	–	(Tiwari and Kim, 2013)

[a] *In vitro* experiments using different culture media are not included.

reduction (99.9%) in *E. coli* O157:H7 numbers (~10^{10} CFU by oral gavage) by using a cocktail of CEV1 and CEV2 (~10^{11} PFU) in sheep (Raya et al., 2011).

Administration of 1.3×10^{11} PFU of KH1 phage orally had no significant effect on bacterial numbers in sheep feces (Table 1). Using high doses of phage preparations increased the phage PFU in animal feces, but did not reduce *E. coli* O157 CFU. The authors proposed that this may be related to the nature of the KH1 phage and/or the conditions in the intestinal tract. However, treatment of mice orally with the SH1 phage alone or in combination with KH1 (~10^{10} PFU), eliminated the *E. coli* O157:H7 bacteria from the feces of mice infected with an oral dose of 10^8 CFU. A high titer cocktail of KH1 and SH1 (~10^{10} PFU) used at the rectoanal junction in cattle decreased *E. coli* O157:H7 CFU as well (Sheng et al., 2006).

Callaway et al. used an oral (~10^{10} PFU) cocktail of 8 phages isolated from feedlot to treat sheep (Table 1). They could reduce the bacterial counts from 10^7 CFU/g feces to 10^4 CFU/g feces, but were not able to eliminate the pathogens completely (Callaway et al., 2008; Callaway et al., 2006).

Using phage e11/2 at MOIs 100 and 1 in a model rumen system inoculated with 10^3 or 10^6 CFU/mL of *E. coli* O157:H7 reduced significantly the bacterial numbers within 1 h. In the same conditions, e4/1c (MOIs 10 and 1000) reduced bacterial count within 2 h. However, oral doses of bacteriophage cocktail of e11/2 and e4/1c (10^{11} PFU) repeated for 3 days could not eliminate *E. coli* O157 from cattle feces (Table 1). Although the model rumen results for these two phages seemed promising, *in vivo* results were not as promising (Rivas et al., 2010). A cocktail of e11/2 (1×10^9 PFU/mL) and e4/1c (1×10^{10} PFU/mL) phages was also tested on 400 cm^2 cattle hide portions having 1×10^6 CFU/cm^2 *E. coli* O157:H7. The results after immediate sampling showed that this treatment was not more effective than washing with water only. However, when sampling was done after 1 h, the bacterial numbers were significantly lower (1.5 $\log_{10}$ CFU/cm^2) than in water washed samples (Coffey et al., 2011).

Rozema et al. used a cocktail of four anti-O157:H7 coliphages (rV5, wV7, wV8, wV11) orally, rectally, and in a combination of both routes (~10^{11} PFU). They found that oral administration of phages was more effective in reducing fecal shedding of *E. coli* O157:H7 than rectal administration (Rozema et al., 2009). Sheng et al. reported the efficacy of rectal administration of phages to steers. They also used a concentration of ~10^6 PFU/mL phages in tested animal drinking water (Sheng et al., 2006). Rosema et al. suggested that oral administration of phages in drinking water may be the reason of effectiveness of rectal administration of phages in the Sheng et al. paper (Rozema et al., 2009). In another study, oral administration of a cocktail of phages SP15, SP21, and SP22 (10^{10} PFU, daily) in mice enhanced the rate of reduction of bacteria in animal feces compared to groups receiving no phage and a single oral phage dose (Table 1) (Tanji et al., 2005).

4. The bacterial surface receptors of anti-O157:H7 phages

The *E. coli* O157:H7 surface receptors that phages use for adsorption have been characterized for some of the phages. LPS and the outer membrane porins are the most common receptors for phage attachment. LPS is the receptor of the KH1, KH4, and KH5 phages, while phages CEV1 and pp01 bind to OmpA and OmpC, respectively (Kudva et al., 1999; Morita et al., 2002). It is proposed that OmpC and LPS are both used by the AR1 phage (Yu et al., 2000). The *E. coli ompC* mutant was resistant to phage SP21 while LPS mutants were shown to be resistant to phage SP22 (Tanji et al., 2004). The ferrichrome-iron receptor FhuA has been identified as the receptor for CEV2 (Raya et al., 2011). The identification of the phage receptors is important since phage resistant mutants are frequently isolated. To prevent this it is recommended to use phages with different receptors in a phage cocktail.

5. Problems and solutions

Gastrointestinal tract of food animals is an anaerobic environment where *E. coli* O157:H7, a facultatively anaerobic organism, can survive and grow, however, under tight competition with the gut normal flora. Bacteriophage proliferation is dependent on its host growth rate and it does not take place in non-growing host cells. Also, the ability of the phages to adapt to, and proliferate, under anaerobic conditions should also be taken under consideration. Indeed, it was shown that aeration was important for the elimination of bacteria by the phages from *in vitro* cultures. In non-aerated samples, bacteria were eliminated only after 5 days and at 4 °C (Kudva et al., 1999; Rivas et al., 2010). Only a few phages have been found to be effective under anaerobic conditions, such as a cocktail of phages EP16, PP17, SP22 that significantly decreased the *E. coli* O157:H7 numbers in anaerobic condition (Kunisaki and Tanji, 2010).

The main problem in oral application of phages is acidity and proteolytic activity of the stomach (Ryan et al., 2011). Most phages are acid-sensitive and cannot tolerate acidic conditions of stomach. Koo et al. used antacid with vibriophages and proposed that antacids increase phage survival in stomach (Koo et al., 2001). Smith et al. used $CaCO_3$ to protect phage from stomach acidity (Smith et al., 1987). Also, the cocktail which Tanji used was not stable at acidic pH, so $CaCO_3$ was used to prepare the cocktail (Tanji et al., 2005). Microencapsulation of phage particles in polymers could also be a suitable method to increase phage viability. Chitosan-Alginate microencapsulated Felix O1 (a *Salmonella* phage) survived much better than free phage under conditions simulating pig gastrointestinal tract (Ma et al., 2008). Stanford et al. used phages rV5, wV7, wV8, and wV11 as a cocktail in methacrylate polymer. The encapsulation was effective in protecting phages from pH of 3 *in vitro*. However, when used in cattle, the encapsulated phages did not reduce *E. coli* O157 shedding in feces, but reduced the shedding period (Stanford et al., 2010). It is recommended in all phage therapy experiments to use a cocktail of phages with different host ranges and different receptors on host to broaden the host range and reduce occurrence of phage resistance in the target organisms (Tanji et al., 2005; Tanji et al., 2004).

6. Conclusions

The isolated phages against pathogenic bacterium *E. coli* O157:H7 were effective in eliminating or decreasing the number of bacteria *in vitro*. However, *in vivo* experiments did not demonstrate such clear efficiency. There are several problems using phages orally and pharmaceutical sciences could introduce novel strategies to solve these problems. The work should be continued as more efficient phage treatment regimens might be found in future. In addition, more studies should be focused on finding active phages in digestive system pH and anaerobic conditions.

Conflict of interest

No conflict of interest declared.

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References

Abuladze, T., Li, M., Menetrez, M.Y., Dean, T., Senecal, A., Sulakvelidze, A., 2008. Bacteriophages reduce experimental contamination of hard surfaces, tomato, spinach, broccoli, and ground beef by *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 74, 6230–6238.

Alam, M., Akhter, M.Z., Yasmin, M., Ahsan, C.R., Nessa, J., 2011. Local bacteriophage isolates showed anti-*Escherichia coli* O157:H7 potency in an experimental ligated rabbit ileal loop model. Can. J. Microbiol. 57, 408–415.

Amarillas, L., Chaidez-Quiroz, C., Sanudo-Barajas, A., Leon-Felix, J., 2013. Complete genome sequence of a polyvalent bacteriophage, phiKP26, active on *Salmonella* and *Escherichia coli*. Arch. Virol. 158, 2395–2398.

Bach, S.J., McAllister, T.A., Veira, D.M., Gannon, V.P.J., Holley, R.A., 2003. Effect of bacteriophage DC22 on *Escherichia coli* O157:H7 in an artificial rumen system (Rusitec) and inoculated sheep. Anim. Res. 52, 89–101.

Boyacioglu, O., Sharma, M., Sulakvelidze, A., Goktepe, I., 2013. Biocontrol of *Escherichia coli* O157:H7 on fresh-cut leafy greens. Bacteriophage 3, e24620.

Bradbury, J., 2004. "My enemy's enemy is my friend." Using phages to fight bacteria. Lancet 363, 624–625.

Callaway, T.R., Edrington, T.S., Brabban, A.D., Anderson, R.C., Rossman, M.L., Engler, M.J., Carr, M.A., Genovese, K.J., Keen, J.E., Looper, M.L., Kutter, E.M., Nisbet, D.J., 2008. Bacteriophage isolated from feedlot cattle can reduce *Escherichia coli* O157:H7 populations in ruminant gastrointestinal tracts. Foodborne Pathog. Dis. 5, 183–191.

Callaway, T.R., Edrington, T.S., Brabban, A.D., Keen, J.E., Anderson, R.C., Rossman, M.L., Engler, M.J., Genovese, K.J., Gwartney, B.L., Reagan, J.O., Poole, T.L., Harvey, R.B., Kutter, E.M., Nisbet, D.J., 2006. Fecal prevalence of *Escherichia coli* O157, *Salmonella*, *Listeria*, and bacteriophage infecting *E. coli* O157:H7 in feedlot cattle in the Southern Plains region of the United States. Foodborne Pathog. Dis. 3, 234–244.

Capparelli, R., Ventimiglia, I., Roperto, S., Fenizia, D., Iannelli, D., 2006. Selection of an *Escherichia coli* O157:H7 bacteriophage for persistence in the circulatory system of mice infected experimentally. Clin. Microbiol. Infect. 12, 248–253.

Carter, C.D., Parks, A., Abuladze, T., Li, M., Woolston, J., Magnone, J., Senecal, A., Kropinski, A.M., Sulakvelidze, A., 2012. Bacteriophage cocktail significantly reduces *Escherichia coli* O157:H7 contamination of lettuce and beef, but does not protect against recontamination. Bacteriophage 2, 178–185.

Coffey, B., Rivas, L., Duffy, G., Coffey, A., Ross, R.P., McAuliffe, O., 2011. Assessment of *Escherichia coli* O157:H7-specific bacteriophages e11/2 and e4/1c in model broth and hide environments. Int. J. Food Microbiol. 147, 188–194.

d'Herelle, F., 1917. About an invisible microbe antagonistic to dysentery bacilli. CR Acad. Sci. 165, 372–375.

Deresinski, S., 2009. Bacteriophage therapy: exploiting smaller fleas. Clin. Infect. Dis. 48, 1096–1101.

Ferens, W.A., Hovde, C.J., 2011. *Escherichia coli* O157:H7: animal reservoir and sources of human infection. Foodborne Pathog. Dis. 8, 465–487.

Ferguson, S., Roberts, C., Handy, E., Sharma, M., 2013. Lytic bacteriophages reduce *Escherichia coli* O157: H7 on fresh cut lettuce introduced through cross-contamination. Bacteriophage 3, e24323.

Galland, J.C., Hyatt, D.R., Crupper, S.S., Acheson, D.W., 2001. Prevalence, antibiotic susceptibility, and diversity of *Escherichia coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. Appl. Environ. Microbiol. 67, 1619–1627.

Goodridge, L., Gallaccio, A., Griffiths, M.W., 2003. Morphological, host range, and genetic characterization of two coliphages. Appl. Environ. Microbiol. 69, 5364–5371.

Hankin, E.H., 1896. L'action bactericide des eaux de la Jumna et du Gange sur le vibrion du cholera. Ann. Inst. Pasteur 10, 511–523 (Paris).

Hong, Y., Pan, Y., Ebner, P.D., 2014. Development of bacteriophage treatments to reduce *E. coli* O157:H7 contamination of beef products and produce. J. Anim. Sci. 92, 1366–1377.

Hudson, J.A., Billington, C., Cornelius, A.J., Wilson, T., On, S.L., Premaratne, A., King, N.J., 2013. Use of a bacteriophage to inactivate *Escherichia coli* O157:H7 on beef. Food Microbiol. 36, 14–21.

Koo, J., Marshall, D.L., DePaola, A., 2001. Antacid increases survival of *Vibrio vulnificus* and *Vibrio vulnificus* phage in a gastrointestinal model. Appl. Environ. Microbiol. 67, 2895–2902.

Kudva, I.T., Jelacic, S., Tarr, P.I., Youderian, P., Hovde, C.J., 1999. Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. Appl. Environ. Microbiol. 65, 3767–3773.

Kunisaki, H., Tanji, Y., 2010. Intercrossing of phage genomes in a phage cocktail and stable coexistence with *Escherichia coli* O157:H7 in anaerobic continuous culture. Appl. Microbiol. Biotechnol. 85, 1533–1540.

Ma, Y., Pacan, J.C., Wang, Q., Xu, Y., Huang, X., Korenevsky, A., Sabour, P.M., 2008. Microencapsulation of bacteriophage felix O1 into chitosan-alginate microspheres for oral delivery. Appl. Environ. Microbiol. 74, 4799–4805.

Magnone, J.P., Marek, P.J., Sulakvelidze, A., Senecal, A.G., 2013. Additive approach for inactivation of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* spp. on contaminated fresh fruits and vegetables using bacteriophage cocktail and produce wash. J. Food Prot. 76, 1336–1341.

Morita, M., Tanji, Y., Mizoguchi, K., Akitsu, T., Kijima, N., Unno, H., 2002. Characterization of a virulent bacteriophage specific for *Escherichia coli* O157:H7 and analysis of its cellular receptor and two tail fiber genes. FEMS Microbiol. Lett. 211, 77–83.

O'Flynn, G., Ross, R.P., Fitzgerald, G.F., Coffey, A., 2004. Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 70, 3417–3424.

Patel, J., Sharma, M., Millner, P., Calaway, T., Singh, M., 2011. Inactivation of *Escherichia coli* O157:H7 attached to spinach harvester blade using bacteriophage. Foodborne Pathog. Dis. 8, 541–546.

Paton, J.C., Paton, A.W., 1998. Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Clin. Microbiol. Rev. 11, 450–479.

Raya, R.R., Oot, R.A., Moore-Maley, B., Wieland, S., Callaway, T.R., Kutter, E.M., Brabban, A.D., 2011. Naturally resident and exogenously applied T4-like and T5-like bacteriophages can reduce *Escherichia coli* O157:H7 levels in sheep guts. Bacteriophage 1, 15–24.

Raya, R.R., Varey, P., Oot, R.A., Dyen, M.R., Callaway, T.R., Edrington, T.S., Kutter, E.M., Brabban, A.D., 2006. Isolation and characterization of a new T-even bacteriophage, CEV1, and determination of its potential to reduce *Escherichia coli* O157:H7 levels in sheep. Appl. Environ. Microbiol. 72, 6405–6410.

Rivas, L., Coffey, B., McAuliffe, O., McDonnell, M.J., Burgess, C.M., Coffey, A., Ross, R.P., Duffy, G., 2010. In vivo and ex vivo evaluations of bacteriophages e11/2 and e4/1c for use in the control of *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 76, 7210–7216.

Rozema, E.A., Stephens, T.P., Bach, S.J., Okine, E.K., Johnson, R.P., Stanford, K., McAllister, T.A., 2009. Oral and rectal administration of bacteriophages for control of *Escherichia coli* O157:H7 in feedlot cattle. J. Food Prot. 72, 241–250.

Ryan, E.M., Gorman, S.P., Donnelly, R.F., Gilmore, B.F., 2011. Recent advances in bacteriophage therapy: how delivery routes, formulation, concentration and timing influence the success of phage therapy. J. Pharm. Pharmacol. 63, 1253–1264.

Schroeder, C.M., Zhao, C., DebRoy, C., Torcolini, J., Zhao, S., White, D.G., Wagner, D.D., McDermott, P.F., Walker, R.D., Meng, J., 2002. Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine, and food. Appl. Environ. Microbiol. 68, 576–581.

Shahrbabak, S.S., Khodabandehlou, Z., Shahverdi, A.R., Skurnik, M., Ackermann, H.W., Varjosalo, M., Yazdi, M.T., Sepehrizadeh, Z., 2013. Isolation, characterization and complete genome sequence of PhaxI: a phage of *Escherichia coli* O157:H7. Microbiology 159, 1629–1638.

Sharma, M., Patel, J.R., Conway, W.S., Ferguson, S., Sulakvelidze, A., 2009. Effectiveness of bacteriophages in reducing *Escherichia coli* O157:H7 on fresh-cut cantaloupes and lettucet. J. Food Prot. 72, 1481–1485.

Sharma, M., Ryu, J.H., Beuchat, L.R., 2005. Inactivation of *Escherichia coli* O157:H7 in biofilm on stainless steel by treatment with an alkaline cleaner and a bacteriophage. J. Appl. Microbiol. 99, 449–459.

Sheng, H., Knecht, H.J., Kudva, I.T., Hovde, C.J., 2006. Application of bacteriophages to control intestinal *Escherichia coli* O157:H7 levels in ruminants. Appl. Environ. Microbiol. 72, 5359–5366.

Smith, H.W., Huggins, M.B., Shaw, K.M., 1987. Factors influencing the survival and multiplication of bacteriophages in calves and in their environment. J. Gen. Microbiol. 133, 1127–1135.

Stanford, K., McAllister, T.A., Niu, Y.D., Stephens, T.P., Mazzocco, A., Waddell, T.E., Johnson, R.P., 2010. Oral delivery systems for encapsulated bacteriophages targeted at *Escherichia coli* O157:H7 in feedlot cattle. J. Food Prot. 73, 1304–1312.

Tanji, Y., Shimada, T., Fukudomi, H., Miyanaga, K., Nakai, Y., Unno, H., 2005. Therapeutic use of phage cocktail for controlling *Escherichia coli* O157:H7 in gastrointestinal tract of mice. J. Biosci. Bioeng. 100, 280–287.

Tanji, Y., Shimada, T., Yoichi, M., Miyanaga, K., Hori, K., Unno, H., 2004. Toward rational control of *Escherichia coli* O157:H7 by a phage cocktail. Appl. Microbiol. Biotechnol. 64, 270–274.

Tiwari, B.R., Kim, J., 2013. Complete genome sequence of bacteriophage EC6, capable of lysing *Escherichia coli* O157:H7. Genome Announc. 1 (e00085-00012).

Twort, F., 1915. An investigation on the nature of ultra-microscopic viruses. Lancet 2, 1241–1243.

Viazis, S., Akhtar, M., Feirtag, J., Brabban, A.D., Diez-Gonzalez, F., 2011a. Isolation and characterization of lytic bacteriophages against enterohaemorrhagic *Escherichia coli*. J. Appl. Microbiol. 110, 1323–1331.

Viazis, S., Akhtar, M., Feirtag, J., Diez-Gonzalez, F., 2011b. Reduction of *Escherichia coli* O157:H7 viability on hard surfaces by treatment with a bacteriophage mixture. Int. J. Food Microbiol. 145, 37–42.

Viazis, S., Akhtar, M., Feirtag, J., Diez-Gonzalez, F., 2011c. Reduction of *Escherichia coli* O157:H7 viability on leafy green vegetables by treatment with a bacteriophage mixture and trans-cinnamaldehyde. Food Microbiol. 28, 149–157.

Wong, C.S., Mooney, J.C., Brandt, J.R., Staples, A.O., Jelacic, S., Boster, D.R., Watkins, S.L., Tarr, P.I., 2012. Risk factors for the hemolytic uremic syndrome in children infected with *Escherichia coli* O157:H7: a multivariable analysis. Clin. Infect. Dis. 55, 33–41.

Yu, S.L., Ko, K.L., Chen, C.S., Chang, Y.C., Syu, W.J., 2000. Characterization of the distal tail fiber locus and determination of the receptor for phage AR1, which specifically infects *Escherichia coli* O157:H7. J. Bacteriol. 182, 5962–5968.

Zuber, S., Ngom-Bru, C., Barretto, C., Bruttin, A., Brussow, H., Denou, E., 2007. Genome analysis of phage JS98 defines a fourth major subgroup of T4-like phages in *Escherichia coli*. J. Bacteriol. 189, 8206–8214.